

TableS2: Strain used in this study

Strain	Description
Spn23F	PMEN1 reference genome (ATCC700669)
R6	lab reference genome (AE007317)
SV35-T23	PMEN1 strain isolated in New York in 1996 (ADNN)
Spn23F RMKO	Restriction enzyme and methylase replaced with spectinomycin resistance cassette
SV35-T23 RMKO	Restriction enzyme and methylase replaced with spectinomycin resistance cassette
Spn23F REKO	Restriction enzyme replaced with spectinomycin resistance cassette
SV3-T235 RE-6His	6His tag added to native RE on chromosome followed by spectinomycin resistance cassette
Spn23F pRE-6His	23F carrying plasmid pRE-6His: a derivative of pLS1-rom expressing RE-6His from a maltose inducible promoter
Spn23F spec	spectinomycin cassette is inserted in between 5,10 methylenetetrahydrofolate reductase (metF-SPN23F05300) and a polyribonucleotide nucleotidyltransferase (pnp-SPN23F05310)
Spain6B spec	spectinomycin cassette is inserted in between 5,10 methylenetetrahydrofolate reductase (metF) and a polyribonucleotide nucleotidyltransferase (pnp)
R6 dpnIII	the entire region coding for the DpnIII system plus a kanamycin cassette is inserted in the BgaA gene.